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## INHERITANCE OF THREE SEED-COAT COLOR GENES IN *VIGNA SINENSIS* SAVI<sup>1</sup>

FRANCIS L. SMITH<sup>2</sup>

SEED-COAT COLOR data for this study were collected during the course of breeding Blackeye cowpeas for cowpea-wilt resistance. This program, however, was conducted in wilt-infested soil, which results in the premature death of a number of plants. Hybrid populations were, therefore, grown in wilt-free soil for the seed-coat color studies.

Only three of the many genes known to be in *Vigna sinensis* were studied in this work. One of these is the black gene, which was denoted by Harland (1919, 1920) as B. The same symbol was used later by Spillman and Sando (1930) to represent brown. Black is dominant to brown. The second gene encountered in this work was described by Spillman (1911) as the Watson pattern from a variety name. The pattern is shown in figure 2. This gene was given the symbol W. The third gene was called Holstein by Spillman and Sando (1930) and was given the symbol H. When heterozygous, this gene was found to give an expression different from either of the homozygotes. The three pattern types shown in figures 3, 4, and 5 are: HH, Holstein true breeding; Hh, large eye heterozygous; and hh, small eye true breeding. The combination of the two dominant genes W and H results in self-colored beans (fig. 1). Thus, neither color-pattern gene is effective unless the other is in the recessive condition. The interactions of these three genes give ten distinguishable color-pattern types:

BB, WW, HH or with any one or combination of two or three heterozygous, self-colored black .....	Fig. 1
bb, WW, HH, or with either W or H or both heterozygous, self-colored brown .....	Fig. 1
BB, WW, hh or with B or W heterozygous, Black Watson .....	Fig. 2
bb, WW, hh or with W heterozygous, Brown Watson .....	Fig. 2
BB, ww, HH or with B heterozygous, Black Holstein .....	Fig. 3
bb, ww, HH, Brown Holstein .....	Fig. 3
BB, ww, Hh or with B heterozygous, Black Large Eye .....	Fig. 4
bb, ww, Hh, Brown Large Eye .....	Fig. 4
BB, ww, hh or with B heterozygous, Black Small Eye .....	Fig. 5
bb, ww, hh, Brown Small Eye .....	Fig. 5

<sup>1</sup> Received for publication February 18, 1955.

<sup>2</sup> Professor of Agronomy, Davis.

## MATERIALS AND METHODS

One of the parents used in these studies was Iron, a self-colored buff variety developed by Webber and Orton (1902). In addition to being resistant to the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, Iron is resistant to cowpea wilt, *Fusarium oxysporum* f. *tracheiphilum* (EFS) Snyder and Hansen. The other parent variety used was Blackeye 5, developed by Mackie (1946) in a backcross breeding program with Iron. Mackie succeeded in transferring resistance to both nematode and cowpea wilt into the blackeye derivative, Blackeye 5. However, the wilt resistance of Blackeye 5 soon began to break down in the field. Presumably, a new physiological form of wilt organism to which Blackeye 5 was not resistant began to increase naturally. A new breeding program was begun to incorporate more resistance from Iron by crossing it with Blackeye 5. The seed-coat color inheritance studies were made from the resulting crosses. Mackie (1946) had noted most of the color reactions but did not study them closely. In figure 13 of his bulletin one can pick out most of the color patterns found in the present study.

## RESULTS

The  $F_1$  plants resulting from the cross Blackeye 5  $\times$  Iron were self-colored black. An occasional bean was mottled, but these were not hereditarily different as they could be found in the same pod with pure black beans (see fig. 1).

The first  $F_2$  population was grown in a wilt-sick soil near Ceres, California. Over 3,000 plants were classified for seed-coat color. The results are shown in table 1. The  $F_2$  results were fitted to expected tri-hybrid ratios of three independent genes. These results were felt to be inadequate because the plants were crowded and some did not even produce a pod. Then, too, all the pattern types were not clearly identified. Too many plants were classified simply as "blackeye" or "brown-eye," so these classes were too large and the larger-eye classes were too small. The results did not fit expectations at all.

In 1949, spaced plantings were made in wide rows in wilt-free soil at Davis. In that year 5,316 plants were classified (table 1). There were too many black and too few brown in all classifications. In some plants it was difficult to distinguish Holstein from large eye. The very poor fit to expected ratios is, however, not due entirely, or even chiefly, to difficulties with these classes.

In 1951 another  $F_2$  population was grown at Davis from the same lot of  $F_1$  seed used in 1948 and 1949. This population of 498 plants, shown in table 1, fits the expected ratios quite well with a  $P$  value of 0.10–0.20.

The  $F_2$  data from table 1 can be broken down into single-gene segregations. In the case of the black gene,  $B$ , the proportion of  $bb$  was too small every year. The  $\chi^2$  value for goodness of fit to a 3:1 ratio was 62.26 in 1948, 31.08 in 1949, and 0.32 in 1951. The  $\chi^2$  for homogeneity was 10.84, giving a  $P$  value of 0.01–0.02. It is interesting that the three populations were not statistically homogeneous even though they were grown from the same lot of seed. The calculated frequency of the recessive  $bb$  in the three years was 0.1889, 0.2169, and 0.2390, and the average was 0.2083. Since the zygotic



TABLE 1  
SUMMARY OF BREEDING RESULTS OF F<sub>2</sub> IN THREE YEARS

Genes and color	F <sub>2</sub> 1948	F <sub>2</sub> 1949	F <sub>2</sub> 1951	Total	$\frac{(O-C)^2}{C}$
BWH: Black self-color:					
Observed.....	1,486	2,563	226	4,275	.....
Calculated.....	1,321.73	2,242.69	210.09	3,774.52	66.36
BWh: Black Watson pattern:					
Observed.....	414	585	55	1,054	.....
Calculated.....	440.58	747.56	70.03	1,258.17	33.13
BwHH: Black Holstein pattern:					
Observed.....	125	261	28	414	.....
Calculated.....	146.86	249.19	23.34	419.39	0.07
BwHh: Black large eye:					
Observed.....	204	503	47	754	.....
Calculated.....	293.72	498.38	46.69	838.78	8.67
Bwhh: Black small eye:					
Observed.....	312	251	23	586	.....
Calculated.....	146.86	249.19	23.34	419.39	66.19
bWH: Brown self-color:					
Observed.....	350	739	83	1,172	.....
Calculated.....	440.58	747.56	70.03	1,258.17	5.90
bWh: Brown Watson pattern:					
Observed.....	84	162	17	263	.....
Calculated.....	146.86	249.19	23.34	419.39	58.32
bwHH: Brown Holstein pattern:					
Observed.....	23	68	4	95	.....
Calculated.....	48.95	83.06	7.78	139.80	14.36
bwHh: Brown large eye:					
Observed.....	68	130	10	208	.....
Calculated.....	97.91	166.12	15.66	279.59	18.33
bwhh: Brown small eye:					
Observed.....	67	54	5	126	.....
Calculated.....	48.95	83.06	7.78	139.80	1.36
Total.....	3,133	5,316	498	8,947	.....
$\chi^2$ .....	313.45	142.78	14.31	470.52	272.59
Probability.....	Very low	Very low	0.10-0.20	Very low	Very low
$\chi^2$ for homogeneity test.....					197.95
Probability.....					< 0.01

frequency of the recessive class should equal the square of the frequency of the recessive gametes in the population, the frequency of the b gene was estimated to be 0.4347, 0.4657, and 0.4889 for the three years and 0.4564 for the total of the F<sub>2</sub> populations.

The data for the Watson pattern gene, W, showed that the homogeneity  $\chi^2$  for the three years was 3.16 with a *P* value of 0.30-0.50. The  $\chi^2$  for goodness of fit to a 3:1 ratio was 0.42 for 1948, 3.86 for 1949, and 0.60 for 1951. The calculated frequency for the recessive class was 0.2550, 0.2383, and 0.2349 for the three years and 0.2440 for the total. This gave a gametic fre-

quency of the *w* gene of 0.5050 in 1948, 0.4882 for 1949, 0.4847 for 1951, and 0.4940 for the total.

The segregation for the Holstein pattern gene, *H*, in the three populations showed  $\chi^2$  values for goodness of fit to the 3:1 ratio of 16.60 in 1948, 76.98 in 1949, and 6.43 in 1951. The  $\chi^2$  value of the homogeneity test of the three populations was 2.03. The *P* value of 0.50–0.70 indicated homogeneity of the three populations. The calculated frequency of the recessive class for the three years was 0.2134, 0.1979, and 0.2008, and the total was 0.2025. The gametic frequency of the *h* gene was 0.4619 in 1948, 0.4449 in 1949, 0.4482 in 1951, and 0.4500 for the total.

These data indicate that there was a paucity of the recessive classes. This could be due to differential gametic competition or to a lower survival of zygotes containing homozygous recessive genes. Some critical information on these alternatives is presented later in this report from studies of the  $F_3$  populations.

**Results from Planting Self-colored Black  $F_2$  Seed.** Of the 226 black-seeded  $F_2$  plants obtained in 1951, 203 were progeny tested in 1952. A maximum of 100 seeds was obtained from each of these 203 plants and planted in 100-foot rows. The 23  $F_2$  plants that were not tested had less than 60 seeds. In order to be self-colored black each plant must have had at least one dominant allele of each gene. The  $F_3$  populations made it possible to assign the genotype of each plant. The results shown in table 2 give a rather poor fit to expected ratios, assuming purely random distribution of the gametes. The probability was between 0.01 and 0.02. The greatest deviation was obtained in the true-breeding blacks where more than twice as many were obtained as expected. This class contributed 9.556 to the total  $\chi^2$  of 17.500.

On the basis of random assortment of gametes one third of the population should breed true for each of the three genes and two thirds should segregate. In a population of 203 plants, then, 67.67 should be homozygous. The number of plants that proved to be homozygous was 79 for *B*, 84 for *W*, and 72 for *H*. The probabilities that these distributions were due to chance were 0.05–0.10 for *B*, 0.01–0.02 for *W*, and 0.50–0.70 for *H*. In all three genes there was a preponderance of the dominant homozygous class. Since all the  $F_2$  plants were phenotypically alike, there was no advantage to a plant whether the gene was homozygous or heterozygous. The poor fit to random segregation, then, can be accounted for by assuming an advantage of the gametes containing dominant alleles.

The  $F_3$  families were planted in May with about 100 seeds per row. Since the land was not preirrigated, germination of the seed was rather poor. Under these severe conditions is there any difference in the size of families that germinated and survived to produce seed? The 79 BB rows averaged 41.1 plants and the 124 Bb rows had an average of 36.3 plants. The difference of 4.8 plants was significant at the 5 per cent level (4.3 required). The 84 WW rows had an average of 38.2 plants and the 119 Ww rows had 38.1. A difference of 4.0 plants was required for significance at the 5 per cent level. The 72 HH rows had 36.2 plants and the 131 Hh rows had 39.2. This difference of 3.0 plants per row was in favor of the heterozygous genotypes, but 4.2 plants were required for a significant difference at the 5 per cent level. Family-size studies, then, demonstrated a difference in favor of the



homozygous genotypes only in the case of B. The evidence does not make a very convincing case for differential survival of phenotypes with recessive genes.

The breeding results of the 203  $F_3$  families grown from black-seeded  $F_2$  plants are shown in table 3.

The families segregating for only one gene, whether B, H, or W, gave a close approximation to a 3:1 ratio. The 21 families segregating for both B and H also fit expectation closely, but the 32 segregating for W and H and the 28 segregating for B and W, as well as the 53 families segregating

TABLE 2  
GENOTYPES OF BLACK SELF-COLORED  $F_2$  PLANTS AS  
DETERMINED BY THEIR  $F_3$  BREEDING RESULTS

$F_2$ Genotypes	Expected ratio	Number of $F_3$ families	Expected number assuming random distribution of gametes
BBWWHH.....	1	16	7.52
BBWWHh.....	2	13	15.04
BBWwHH.....	2	18	15.04
BbWWHH.....	2	22	15.04
BBWwHh.....	4	32	30.07
BbWWHh.....	4	21	30.07
BbWwHH.....	4	28	30.07
BbWwHh.....	8	53	60.15
Total.....	..	203	203.00
$\chi^2$ .....	..	..	17.50
$P$ .....	..	..	0.01-0.02

for all three genes, gave very low  $P$  values when fitted to expected ratios. The 53 families were genetically like the  $F_2$  in which poor fits were obtained.

Breeding results from all possible genotypes have been obtained. These will now be presented.

**Results from Backcrosses.** A number of  $F_1$  plants of the cross Blackeye  $5 \times$  Iron were backcrossed to the Blackeye parent. This backcross was BbWwHh  $\times$  BBwwhh. Unfortunately, the difference between large and small eyes was not appreciated when the counts were made, so all eyed patterns were grouped together. Since B was homozygous in one parent and heterozygous in the other, all the progeny were black as expected.

The backcross population of 414 plants segregated 106 self-colored black, BWH; 103 black Watson pattern, BWh; and 205 blackeyed, BwHh and Bwh. These results fit a 1:1:2 ratio with a  $P$  value between 0.70 and 0.80.

**Breeding Results from Planting  $F_2$  Seed with the Watson Pattern.** The results from the Watson pattern are shown in table 4. Nineteen  $F_3$  families segregating for Bb and Ww gave a good fit to expected ratios ( $P=0.20$ ), although there was a deficiency of ww—21.27 per cent instead of 25 per cent. Fourteen families segregated for ww. Although the fit was good ( $P=0.20-0.30$ ), the ww class was only 21.80 per cent. In eight families segregating for Bb only, the fit was good, with a  $P$  value of 0.80-0.90. Four families bred true. There were 22 families of brown Watson segregating

TABLE 3

SUMMARY OF BREEDING RESULTS OF F<sub>3</sub> FAMILIES FROM BLACK F<sub>2</sub> PLANTS, 1952

F <sub>3</sub> Colors	F <sub>2</sub> Genotypes						
	BBWWHH 16 families	BBWWHh 13 families	BBWWHh 18 families	BbWWHH 22 families	BBWwHh 32 families	BbWWHh 21 families	BbWwHh 28 families
	Number of plants						
Black self-color:							
Observed	642	427	590	526	737	404	610
Calculated	...	493.00	598.50	525.75	700.88	394.31	602.44
Black Watson pattern:							
Observed	...	137	...	...	190	117	...
Calculated	...	141.00	...	...	233.62	131.44	...
Black Holstein pattern:							
Observed	...	...	208	...	111	...	212
Calculated	...	...	199.50	...	77.88	...	200.81
Black large eye:							
Observed	...	...	...	...	143	...	...
Calculated	...	...	...	...	155.75	...	...
Black small eye:							
Observed	...	...	...	...	65	...	...
Calculated	...	...	...	...	77.87	...	...
Brown self-color:							
Observed	...	...	...	175	...	136	207
Calculated	...	...	...	175.95	...	131.44	200.81
Brown Watson pattern:							
Observed	...	...	...	...	...	44	...
Calculated	...	...	...	...	...	43.81	...
Brown Holstein pattern:							
Observed	...	...	...	...	...	...	...
Calculated	...	...	...	...	...	...	...
Brown large eye:							
Observed	...	...	...	...	...	...	...
Calculated	...	...	...	...	...	...	...
Brown small eye:							
Observed	...	...	...	...	...	...	...
Calculated	...	...	...	...	...	...	...
Total	642	564	798	701	1,246	701	1,071
$\chi^2$	...	0.151	0.483	0.001	27.271	1.983	10.199
P	...	0.50-0.70	0.30-0.50	0.98-0.99	Very low	0.50-0.70	0.01-0.02
							< 0.01



for Ww, with a *P* value of 0.70–0.80, but the ww class was 24.22 per cent. Six brown Watson families bred true.

These results indicate that the Watson pattern was behaving in a normal manner except for a deficiency in the recessive class as was also found in *F*<sub>2</sub>. The deficiencies in *F*<sub>3</sub> families did not cause low *P* values because the families were relatively small.

Since the results shown in table 4 are from *F*<sub>3</sub> progenies, it is possible to fit the *F*<sub>2</sub> genotypes to expected proportions. The 45 black Watson progenies tested can be classified as 19 segregating for B and W, 14 segregating for W, 8 segregating for B, and 4 breeding true. This should fit a 4:2:2:1 ratio. The  $\chi^2$  for such a fit is 2.25, giving a *P* value of 0.50–0.70. Similarly, the 28 brown Watson families should be in the ratio of 2 segregating for W to 1 breeding true. The  $\chi^2$  is 1.78 and the *P* value is 0.20–0.30. The data from the *F*<sub>3</sub> of the Watson pattern confirm the hypothesis that

TABLE 4

SUMMARY OF *F*<sub>3</sub> BREEDING RESULTS FROM THE WATSON PATTERN

Genotype	Number of families	Number of progeny					$\chi^2$	<i>P</i>
		Black		Brown		Total		
		Watson	Small eye	Watson	Small eye			
BbWwhh.....	19	349	98	121	29	597	4.838	0.20
BBWwhh.....	14	226	63	..	..	298	1.579	0.20-0.30
BbWWhh.....	8	121	..	42	..	163	0.051	0.80-0.90
BBWWhh.....	4	79	..	..	..	79	.....	.....
bbWwhh.....	22	..	..	244	78	322	0.104	0.70-0.80
bbWWhh.....	6	..	..	78	..	78	.....	.....

this pattern is due to a single dominant gene, W, which acts only in the presence of homozygous hh. Seed with the Watson pattern can be black or brown depending on the presence of B or b.

**Breeding Results from Planting *F*<sub>2</sub> Seed with the Holstein Pattern.** The Holstein pattern must breed true for the pattern and may segregate for color. Of seven *F*<sub>3</sub> families of black Holstein, one bred true and six segregated for black and brown Holstein. The observed numbers were 70:17. These results, fitted to a 3:1 ratio, gave a  $\chi^2$  value of 1.384 and a probability of 0.20–0.30. The ratio of 6 segregating to 1 breeding true fits a 2:1 ratio with a  $\chi^2$  value of 1.138 and a *P* value of 0.20–0.30. Twelve progenies from brown Holstein bred true (132 plants) as expected. All the results on this pattern fit the hypothesis that Holstein is due to the interaction of wwHH.

**Breeding Results from Planting *F*<sub>2</sub> Seed with the Large-Eye Pattern.** On the basis of the literature and the *F*<sub>2</sub> results, it was supposed that the large-eye pattern was due to the interaction of ww and Hh. All large-eye patterns should therefore segregate into Holstein, large eye, and small eye. Results from progeny tests of both black and brown large-eye types are shown in table 5. They fit expectations very well. Two genotypes of the black large eye are expected, those segregating for both Bb and Hh and those segregating for only Hh. They should be in the ratio of 2:1. Twenty-

nine families were segregating, 17 for B and H and 12 for H only. Fitted to expectation, the  $\chi^2$  value is 0.842 and the  $P$  value is 0.30–0.50. The brown large eye should segregate for Hh but breed true for the color. The 42 families tested did that. Thus, the tests from this pattern behaved as was expected in every particular.

**Breeding Results from Planting  $F_2$  Seed with the Small-Eye Pattern.** This pattern is due to the interaction of the double recessive, ww $hh$ , and should therefore breed true for the pattern type. Black small eye may be homozygous or heterozygous for B. Twelve progeny tests were made from

TABLE 5  
SUMMARY OF  $F_3$  BREEDING RESULTS FROM THE LARGE-EYE PATTERN

Genotype	Number of families	Number of progeny							$\chi^2$	$P$
		Black			Brown			Total		
		Holstein	Large eye	Small eye	Holstein	Large eye	Small eye			
BbwwHh.....	17	26	55	19	10	20	10	140	2.738	0.70-0.80
BBwwHh.....	12	39	84	43	..	..	..	166	0.217	0.90
bbwwHh.....	42	..	..	..	131	245	112	488	1.488	0.20-0.30

TABLE 6  
SUMMARY OF BREEDING RESULTS FROM SELF-COLOR BROWN

Genotype	Number of families	Number of progeny						$\chi^2$	$P$
		Self colored	Watson	Holstein	Large eye	Small eye	Total		
bbWWHH.....	2	22	..	..	..	..	22	.....	.....
bbWWHh.....	3	14	5	..	..	..	19	0.017	0.90
bbWwHH.....	4	47	..	11	..	..	58	1.126	0.20–0.30
bbWwHh.....	11	93	16	10	18	11	148	6.523	0.10–0.20

black small eye in the  $F_2$  following a backcross to the Blackeye variety. The expected ratio should be 1BB:1Bb, and exactly that was obtained—6:6. In the six progenies breeding true, 84 plants were counted. In the six progenies segregating, there were 50 black and 15 brown. These results fit expectation with a  $P$  value of 0.70–0.80. Six progenies have been tested from brown small eye, and they all bred true as expected. The results then from later generations agree in every particular with the literature and the  $F_2$  results.

**Breeding Results from Planting Self-colored Brown  $F_2$  Seed.** This color type is the result of the interaction of bWH. Plants of this genetic constitution should breed true for color but can segregate for either or both of the pattern genes. Five patterns can appear. The results of progeny tests from 20  $F_3$  plants are shown in table 6. The distribution of color types in each of the four groups fits expectation very well. The distribution of the  $F_2$  genotypes as shown in the  $F_3$  should be 1bbWWHH:2bbWWHh:2bbWwHH:4bbWwHh. The fit to these expectations gave a  $\chi^2$  value of 1.036 and a  $P$  value between 0.30 and 0.50.



**Summary of  $F_3$  Breeding Results.** All the possible genotypes have been obtained in the course of these studies. The results are summarized in table 7. This table is arranged in increasing order from 0 to 3 segregating genes. In the 12 genotypes segregating for only one gene the  $P$  values were all above 0.20. Where two genes were segregating simultaneously, two cases of

TABLE 7

ALL POSSIBLE GENOTYPES FOR THREE GENES IN THE CROSS  
BLACK EYE 5  $\times$  IRON AND THE BREEDING RESULTS OBTAINED

Number of genes segregating	Genotypes	Color and pattern	Value of $P$
0	BBWWHH.....	Black self-color	.....
	BBWWhh.....	Black Watson	.....
	BBwwHH.....	Black Holstein	.....
	BBwwhh.....	Black small eye	.....
	bbWWHH.....	Brown self-color	.....
	bbWWhh.....	Brown Watson	.....
	bbwwHH.....	Brown Holstein	.....
	bbwwhh.....	Brown small eye	.....
1	BBWWHh.....	Black self-color	0.50-0.70
	BBWwHH.....	Black self-color	0.30-0.50
	BbWWHH.....	Black self-color	0.98-0.99
	BBWwhh.....	Black Watson	0.20-0.30
	BbWWhh.....	Black Watson	0.80-0.90
	BbwwHH.....	Black Holstein	0.20-0.30
	BBwwHh.....	Black large eye	0.90
	Bbwwhh.....	Black small eye	0.70-0.80
	bbWWHh.....	Brown self-color	0.90
	bbWwHH.....	Brown self-color	0.20-0.30
	bbWwhh.....	Brown Watson	0.70-0.80
	bbwwHh.....	Brown large eye	0.20-0.30
2	BBWwHh.....	Black self-color*	Very low
	BBWwHh.....	Black self-color†	0.70-0.80
	BbWwHH.....	Black self-color	0.01-0.02
	BbWWHh.....	Black self-color	0.50-0.70
	BbWwhh.....	Black Watson	0.20
	BbwwHh.....	Black large eye	0.70-0.80
	bbWwHh.....	Brown self-color	0.10-0.20
3	BbWwHh.....	Black self-color, $F_2$ 1948	Very low
		$F_2$ 1949	Very low
		$F_2$ 1951	0.10-0.20
		$F_3$ 1952	< 0.01

\* Data taken from  $F_2$ .

† Data taken from  $F_1BC_1$ .

low probability were found. The first one was from black  $F_2$  plants segregating for W and H. The same two genes were segregating in a backcross population where a good fit to expectation was obtained. Simultaneous segregation of W and H was also tested in brown self-color where the probability was 0.10-0.20. Thus, in three cases of these two genes segregating together, only one fits expectations poorly. Another poor fit was obtained from black  $F_2$  plants segregating for B and W ( $P=0.01-0.02$ ). The same genes were segregating in some black Watson progeny tests where the  $P$  value was 0.20.



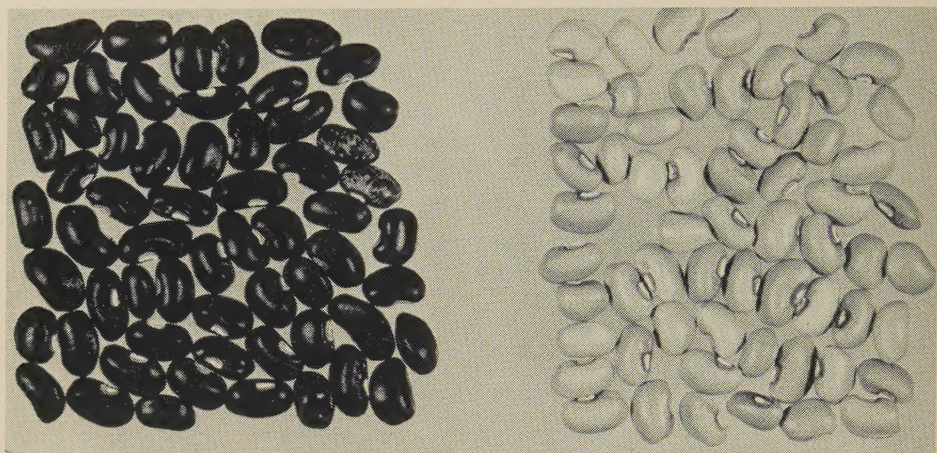


Fig. 1. Left, black, self-color (BWH) ; right, brown, self-color (bWH).

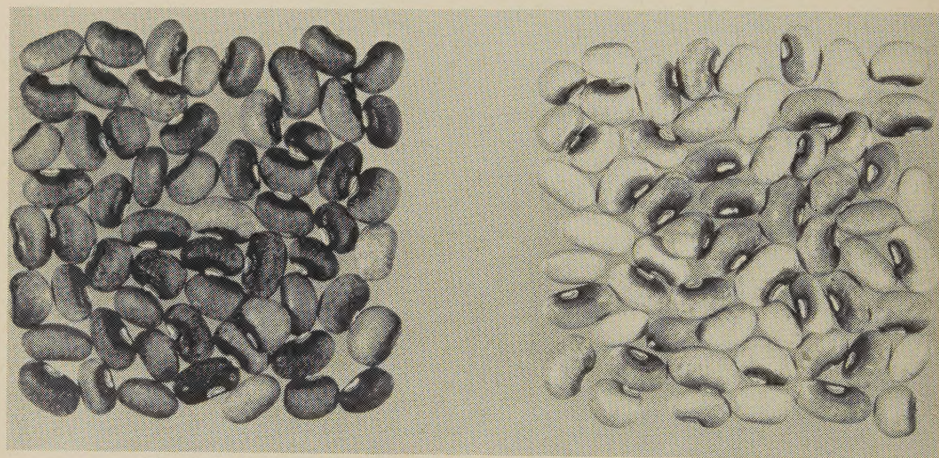


Fig. 2. Left, black Watson (BWh) ; right, brown Watson (bWh).

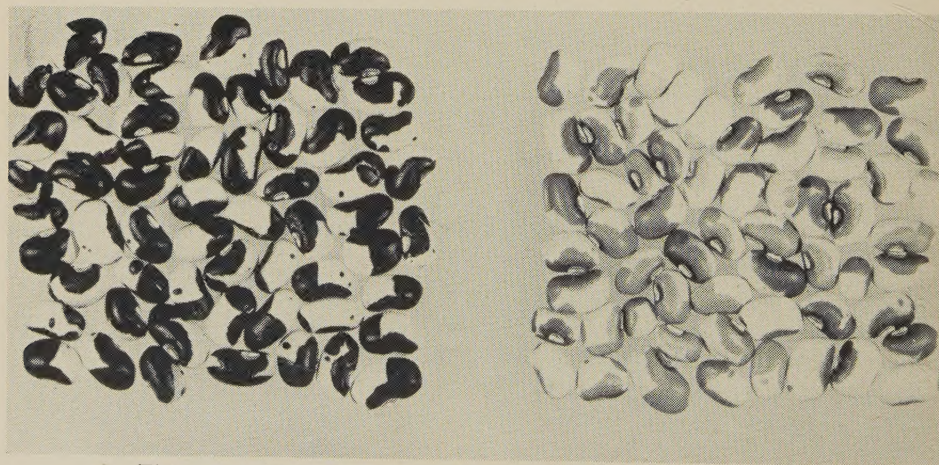


Fig. 3. Left, black Holstein (BwH) ; right, brown Holstein (bwH).



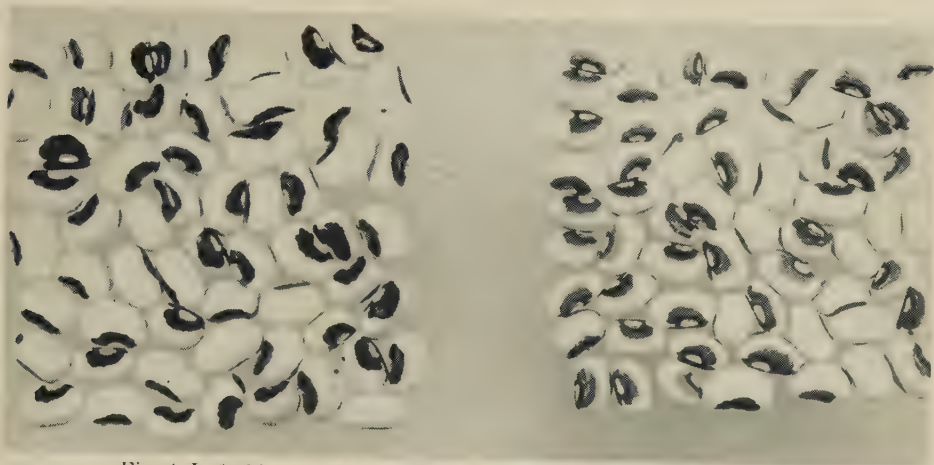


Fig. 4. Left, black large eye (BwHh) ; right, brown large eye (bwhh).

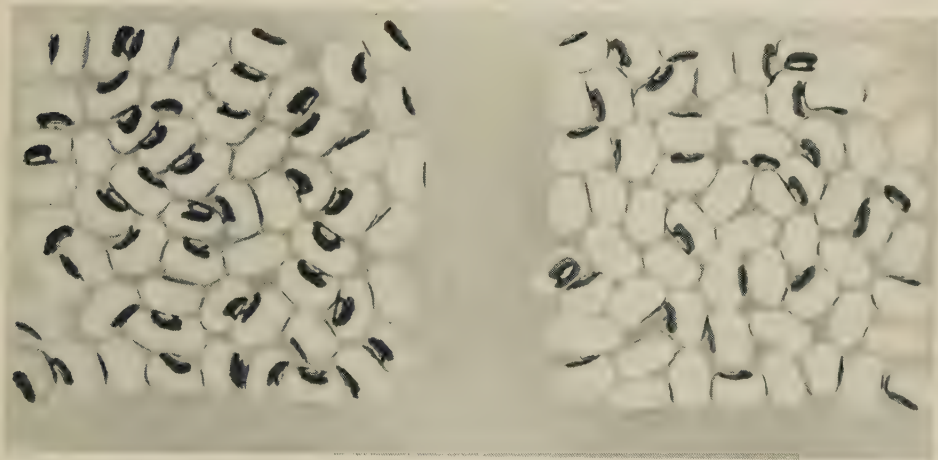


Fig. 5. Left, black small eye (Bwh) ; right, brown small eye (bwh).

In the populations where all three genes were segregating, most of the results deviated markedly from expectations. Only in the  $F_2$  population of 1951, where the number was only 498, was a plausible fit obtained. Two other  $F_2$  populations and a total of 53  $F_3$  families with triple segregation gave very poor fits. These have been discussed earlier. An attempt to explain these results seems in order. It should be pointed out that these populations are the largest, aggregating a total of 10,972 plants. If there are consistent small deviations in segregation, it would be more readily detected in a large population.

**Possible Linkage.** One possible explanation not to be overlooked is that of linkage. Of the three genes in question, B came from one parent and W and H from the other. The fact to be explained is the superabundance of self-colored black in the  $F_2$ , which is due to the presence of all three dominant genes. Linkage of B with either W or H would not provide the answer. Attempts were made to find a possible linkage between W and H from the available data. Such a linkage does not seem probable. Taking the  $F_2$  data from 1949 and 1951, there were 3,611 WH, 819 Wh, 361 wHH, 690 wHh, and 33 wh. With independent assortment the ratios should be 9:3:1:2:1. If there were linkage between W and H, an excess of the WH, wHh, and whh types and a scarcity of Wh and wHH would be expected. This was not the case.

The critical classes are Holstein, wwHH, and small eye, ww hh. If there were linkage between W and H, the Holstein class would be smaller and the small-eye class larger than expected for independent assortment. This did not occur. Therefore, linkage does not seem to be the explanation.

**Differential Advantage of Dominant Alleles.** It is now possible to test the hypothesis expressed earlier that gametes with dominant alleles have a slight advantage in fertilization over those with recessive alleles. For this study the data for the segregation of each of the three genes can be assembled from all the  $F_3$  populations. There were nine genotypes segregating for B, four black self-colored, one black large eye, two black Watson, one black Holstein, and one black small eye. The figures have been given above and need not be repeated in detail. The total of these populations is 5,550 of which 4,209 were black and 1,341 were brown. The probability of these data fitting a 3:1 ratio is 0.10–0.20. The homogeneity  $\chi^2$  of the nine populations was 5.276 with a  $P$  value of 0.80–0.90. It was assumed, therefore, that the populations were homogeneous. The proportion of plants with the bb constitution was 0.24162, the square root of which is 0.4916. This is the calculated frequency of effective gametes containing the b allele.

There were nine genotypes segregating for W in the  $F_3$ . There were four black self-color, two black Watson, two brown self-color, and one brown Watson. The total number of plants in the nine populations was 6,554 of which 5,005 were W and 1,549 were ww. The  $\chi^2$  for the homogeneity test was 10.853 with a  $P$  value of 0.20–0.30. For a 3:1 ratio, 1,638.5 ww plants were expected. The probability of these data fitting such a ratio is 0.01. The proportion of plants with the ww constitution was 0.24076, the square root of which is 0.4862. This is the calculated frequency of effective gametes containing the w allele.

The H gene was segregating in nine  $F_3$  genotypes: four black self-color,



two black large eye, two brown self-color, and one brown large eye. The nine populations had a homogeneity  $\chi^2$  of 6.712 with a probability of 0.50–0.70. The total of all populations was 5,497 of which 1,229 were hh, where 1,374.25 were expected for a 3:1 ratio. The  $\chi^2$  of such results fitting a 3:1 ratio was 20.469 with a probability of considerably below 0.01. The proportion of the hh plants was 0.22358, and the frequency of the effective gametes containing the h allele was 0.4728.

The analysis of the segregation of each gene in the  $F_2$  generation has already been discussed. The data from the two generations can now be combined.

In the Bb segregations the data are:

	B	bb	frequency of b
$F_2$ , 3 populations .....	7,083	1,864	0.4564
$F_3$ , 9 genotypes .....	4,209	1,341	0.4916
Total .....	11,292	3,205	0.4702

It was pointed out earlier that the three  $F_2$  populations were not homogeneous for the Bb segregation. The value of  $P$  of the homogeneity  $\chi^2$  was 0.01–0.02. The same result was obtained when the  $F_2$  and  $F_3$  results were compared. The homogeneity  $\chi^2$  value is 10.24 with a probability of somewhat less than 0.01. The nine genotypes, however, were statistically homogeneous with a  $P$  value of 0.80–0.90. Therefore the 1948  $F_2$  population must be too low. In that year the frequency of the b allele was 0.4347. There is now a choice of arbitrarily deleting some nonconforming data or using all of it. It seems that the estimation of the frequency of the b allele would be better from all the data than from only a part. Therefore, the figure 0.4702 was used with the knowledge that this estimate might be a little low.

For the Ww segregation the data were as follows:

	W	ww	frequency of w
$F_2$ , 3 populations .....	6,764	2,183	0.4940
$F_3$ , 9 genotypes .....	5,005	1,549	0.4862
Total .....	11,769	3,732	0.4907

All the tests for homogeneity indicated the populations were statistically homogeneous. The figure 0.4907 calculated from all the data then seems to be a good estimate of the frequency of the w allele.

The populations segregating for Hh in  $F_2$  and  $F_3$  were:

	H	hh	frequency of h
$F_2$ , 3 populations .....	6,500	1,650	0.4500
$F_3$ , 9 genotypes .....	4,268	1,229	0.4728
Total .....	10,768	2,879	0.4593

The homogeneity tests of the three  $F_2$  populations and the nine  $F_3$  genotypes each gave  $P$  values 0.50–0.70. However, the frequencies of h in the  $F_2$  and  $F_3$  data were not close, and it is little wonder that in combining the two groups of data, the  $P$  value of the homogeneity test was reduced to 0.01.

As in the case of *b*, the figure calculated from all the data was used because it should be more reliable than any part. The calculated proportions for the *B* and the *H* gene in different populations were divergent, as shown by the homogeneity tests. It seems, therefore, that the advantage of the dominant alleles over the recessive is not the same in all populations. Some of the divergence may have been due to sampling errors.

**Tests of the Hypothesis of Greater Efficiency of Dominant over Recessive Alleles.** Now if the frequency of *b* was 0.4702, instead of a 0.50:0.50 ratio of the dominant:recessive, we would expect 0.5298:0.4702. For *W:w* the ratio was 0.5093:0.4907, and for *H:h* it was 0.5407:0.4593. These are but slight changes and would not deviate from the normal 50:50 enough to be detected in small populations. However, if the gametes containing the dominant alleles are favored, it would be expected that the substitution of these figures for the normal ratios would improve the fit in large populations. The  $F_2$  populations would be a good place to test the hypothesis. The  $F_2$  generation of 1948, however, was in error because of faulty classification of some of the patterns. It will therefore not be considered.

In table 8 the results of the  $F_2$  in 1949 are given. The first column lists the genetic symbols for each phenotype. In the second column the observed numbers for each class are given. In the third column, "Calculation 1," the deviations from expectation are given. This calculation is based on equal gametic competition; hence, this is testing to a 27:9:3:6:3:9:3:1:2:1 ratio. The deviation from the calculated number is given for each cell. Deviations large enough to reduce *P* to 0.05 are starred. Those large enough to reduce it to 0.01 are marked with a dagger. In the 1949 data there were six cells where the deviations were large enough to reduce the probability to less than 0.01. The total  $\chi^2$  is 142.8 and the probability is very low. The same calculations were used in determining the goodness of fit in table 1.

In the "Calculation 2" column the calculations were based on unequal competition of gametes containing dominant and recessive alleles. The calculations used to obtain the expected frequencies of the phenotypes were:

$$\begin{aligned} &0.5298^2 + 2(0.5298 \times 0.4702) + 0.4702^2 \text{ for } BB + 2Bb + bb \\ &0.5093^2 + 2(0.5093 \times 0.4907) + 0.4907^2 \text{ for } WW + 2Ww + ww \\ &0.5407^2 + 2(0.5407 \times 0.4593) + 0.4593^2 \text{ for } HH + 2Hh + hh. \end{aligned}$$

It should be noted here that these calculations were based on the assumption of discrimination in favor of the gametes containing dominant alleles. In a self-pollinated plant it would be extremely difficult to determine whether this discrimination is due to pollen on egg cells or to slightly favored dominant combinations in fertilization.

Since the genetic constitution of each phenotypic class was known, the calculated frequency for each cell was made. The numbers obtained by this method were calculated and the deviations of the observed from calculated numbers are shown for each cell in the table in the "Calculated 2" column.

When the data were fitted to expectation from the model assuming unequal gametic competition, there were only two cells in which the deviations were large enough to reduce the probability to 0.01. The total  $\chi^2$  was 32.7 and the probability was near 0.01. The hypothesis assuming unequal competi-



TABLE 8  
COMPARISON OF  $F_2$  DATA WITH EXPECTED NUMBERS  
CALCULATED FOR EQUAL AND UNEQUAL EFFICIENCY  
OF THE DOMINANT AND RECESSIVE ALLELES

Phenotype	Observed	Deviations	
		Calc. 1	Calc. 2
1949			
BWH.....	2,563	320.31†	82.41
BWh.....	585	-162.56†	-78.22†
BwHH.....	261	11.81	-30.45
BwHh.....	503	4.63	7.84
Bwh.....	251	1.81	40.67†
bWH.....	739	-85.63†	34.94
bWh.....	162	-87.19†	-26.24*
bwHH.....	68	-15.06	-14.72
bwHh.....	130	-36.12†	-10.54
bwh.....	54	-29.06†	-5.69
Total.....	5,316	.....	.....
$\chi^2$ .....	.....	142.782	32.740
P.....	.....	Very low	0.01
1951			
BWH.....	226	15.91	-6.38
BWh.....	55	-15.03	-7.13
BwHH.....	28	4.66	0.70
BwHh.....	47	-0.31	0.61
Bwh.....	23	-0.34	3.30
bWH.....	83	12.97	17.04
bWh.....	17	-6.34	-0.63
bwHH.....	4	-3.78	-3.75
bwHh.....	10	-5.56	-3.17
bwh.....	5	-2.78	-0.59
Total.....	498	.....	.....
$\chi^2$ .....	.....	14.311	9.637
P.....	.....	0.10-0.20	0.30-0.50

\* Deviation  $^2$ /calculated has a P value of <0.05.

† Deviation  $^2$ /calculated has a P value of <0.01.

tion of gametes fits the facts more nearly than the one assuming equal effectiveness.

In the lower part of the table the same comparison was made with the  $F_2$  data of 1951. In this case the hypothesis reduced the  $\chi^2$  from 14.3 to 9.6 and the probability increased from 0.10-0.20 to 0.30-0.50.

In table 9 the hypothesis was tested for the 53  $F_3$  families segregating for all three genes. In the calculations assuming equal efficiency of dominant and recessive alleles, there were two cells with very high  $\chi^2$  values and one with a high value. The total  $\chi^2$  was 27.5. These data were given in table 3. When fitted to the model assuming unequal competition of genes, there

was only one cell with a high  $\chi^2$  and the total was 17.1. The probability of goodness of fit was improved from 0.01 to 0.02–0.05.

In the center part of table 9 the 28 families segregating for BbWwHH are shown. These data are also in table 3. The unequal gametic efficiency hypothesis showed a slight improvement over the equal; the value of  $P$  for goodness of fit was raised from 0.01–0.02 to 0.02–0.05.

TABLE 9  
COMPARISON OF  $F_3$  FAMILIES WITH EXPECTED NUMBERS  
CALCULATED FOR EQUAL AND UNEQUAL EFFICIENCY  
OF THE DOMINANT AND RECESSIVE ALLELES

Phenotype	Observed	Deviations from	
		Calc. 1	Calc. 2
53 F <sub>3</sub> families segregating for BbWwHh			
BWH.....	939	84.70†	–5.92
BWh.....	282	–2.77	29.36
BwHH.....	104	9.08	–7.02
BwHh.....	154	–35.84†	–34.62*
Bwh.....	73	–21.92*	–7.12
bWH.....	269	–15.77	0.81
bwh.....	85	–9.92	13.29
bwHH.....	32	0.36	0.49
bwHh.....	67	3.72	13.47
bwh.....	20	–11.64*	–2.74
Total.....	2,025	.....	.....
χ <sup>2</sup> .....	...	27.536	17.073
P.....	...	0.01	0.02–0.25
28 F <sub>3</sub> families segregating for BbWwHH			
BWH.....	610	7.56	–23.38
BWh.....	212	11.19	11.15
bWH.....	207	6.19	27.23*
bWh.....	42	–24.94†	–15.00*
Total.....	1,071	.....	.....
χ <sup>2</sup> .....	...	10.119	9.546
P.....	...	0.01–0.02	0.02–0.05
32 F <sub>3</sub> families segregating BBWwHh			
BWH.....	737	36.13	–9.44
BWh.....	190	–43.62†	–9.57
BwHH.....	111	33.13†	23.30*
BwHh.....	143	–12.75	–6.00
Bwhh.....	65	–12.87	1.71
Total.....	1,246	.....	.....
χ <sup>2</sup> .....	...	27.271	7.003
P.....	...	0.01	0.10–0.20

\* Deviation <sup>2</sup>/calculated has a  $P$  value of <0.05.

† Deviation <sup>2</sup>/calculated has a  $P$  value of <0.01.



In the bottom of table 9 the results of the 32 families segregating for BBWwHh are shown. Here again, the proposed hypothesis fits the results better than the assumption of equality of all gametes. The  $\chi^2$  was reduced from 27.3 to 7.0, and the probability of goodness of fit increased from less than 0.01 to 0.10–0.20.

Most critical are the data dealing with distribution of the 203 F<sub>2</sub> genotypes as determined by their F<sub>3</sub> breeding behavior. These results are given in table 2. There were far too many triple dominant homozygous types. In

TABLE 10  
COMPARISON OF F<sub>2</sub> GENOTYPES AS DETERMINED BY  
THEIR F<sub>3</sub> BREEDING BEHAVIOR WITH EXPECTED  
NUMBERS CALCULATED FOR EQUAL AND  
UNEQUAL EFFICIENCY OF THE DOMINANT  
AND RECESSIVE ALLELES

Phenotype	Observed	Deviations from	
		Calc. 1	Calc. 2
BBWWHH.....	16	8.48†	6.74*
BBWWHh.....	13	-2.04	-2.73
BBWwHH.....	18	2.96	0.16
BbWWHH.....	22	6.96	5.56
BBWwHh.....	32	1.93	1.68
BbWWHh.....	21	-9.05	-6.93
BbWwHH.....	28	-2.07	-3.67
BbWwHh.....	53	-7.15	-0.81
Total.....	203	.....	.....
$\chi^2$ .....	..	17.499	9.504
P.....	..	0.01–0.02	0.30–0.50

\* Deviation  $\chi^2$ /calculated has a *P* value of <0.05.

† Deviation  $\chi^2$ /calculated has a *P* value of <0.01.

table 10 the hypothesis is tested on these genotypes. In the calculations for goodness of fit for equal efficiency of gametes, the  $\chi^2$  was 17.5 and the value of *P* was 0.01–0.02. Fitted to the hypothesis of inequality of efficiency of gametes containing dominant and recessive alleles, the  $\chi^2$  of goodness of fit was reduced to 9.5 and the probability increased to 0.30–0.50. In all but two cells the deviations from the observed were less when the model of this hypothesis was used. This test is significant because the genotypes of each of the 203 F<sub>2</sub> plants were proved in the F<sub>3</sub> progeny tests. Since the exact genotypes of these 203 F<sub>2</sub> plants were established, deviations from normal distribution of the gametes need some explanation. The hypothesis of unequal efficiency of dominant and recessive alleles fitted the results much better than the one assuming random distribution of the gametes containing these alleles. The model for this hypothesis was derived from data obtained from other populations in which zygotic elimination of recessive types would be equally valid. The fact that the model satisfactorily explained the results on this more critical material favors this hypothesis as an explanation for the other results as well.

The hypothesis of unequal efficiency of gametes containing dominant

and recessive alleles explains not only the deficient recessive classes but also the excessive proportions of the  $F_3$  families from black  $F_2$  plants that were homozygous for all three genes. It also explains the phenomenon of exceedingly poor fits to normal distribution obtained in plants that were segregating for three genes.

### SUMMARY

The segregation of three seed-coat color and pattern genes was studied in the cross Blackeye 5  $\times$  Iron. The color gene, B, produces black beans. It is completely dominant to b, which causes the beans to be brown. With the Watson gene, W, the color is distributed over most of the seed coat but concentrated more strongly around the hilum. The color is in fine dots, giving the beans a dirty appearance. The color is black with B and brown with b. The Holstein gene, H, restricts the color either black or brown to about half the seed coat with occasional islands of color in the white area. If both W and H are present, the beans are self-colored; if both w and h are present, the color is restricted to a small area about the hilum. When in the heterozygous condition, Hh, the beans are intermediate between the Holstein pattern and small eye. This pattern is called large eye.

There was no evidence of linkage of the three genes. All the possible genotypes have been studied in the  $F_3$  generation. The data fitted expectations very closely in genotypes segregating for one or two genes and in relatively small families. However, in large populations of genotypes segregating for three genes, significant deviations from expectations were found. There was a slight deficiency in the homozygous recessive classes and too many triple dominants. From all the  $F_2$  and  $F_3$  data it was shown that the recessive classes were deficient in number. Competitive elimination of homozygous recessives did not explain the results of genotype analyses of  $F_2$  plants. A hypothesis of unequal efficiency of gametes containing dominant and recessive alleles was made. Instead of a 0.50:0.50 distribution of dominant to recessive gametes, in the case of B:b it was 0.5298:0.4702; for W:w it was 0.5093:0.4907; and for H:h it was 0.5407:0.4593. When these figures were applied to recalculate expectations of aberrant and large populations, in every case the probabilities of the goodness of fit were improved. A slight difference in efficiency of gametes containing dominant and recessive alleles was therefore assumed.

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